

Dissecting the mechanism of our internal clock: how living organisms tune in to the time of day

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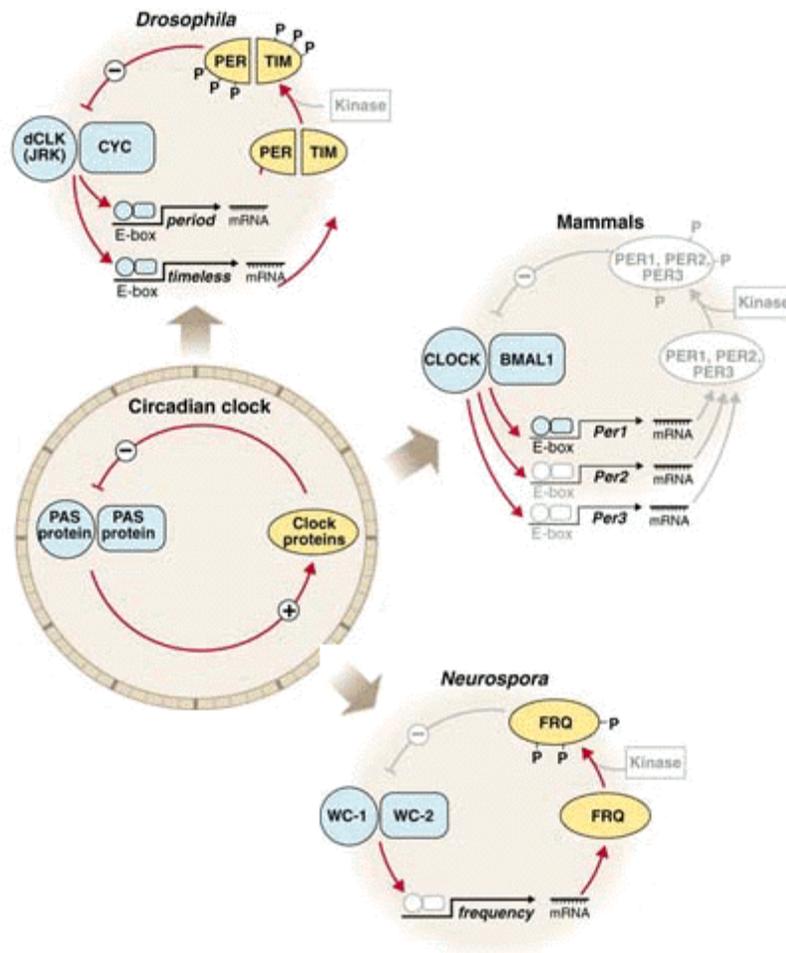
As any jet-setter knows, it takes time to adapt to the shifted day-night cycle of a foreign time zone. We have an internal circadian clock that times many physiological and behavioral events on a 24-hour cycle, according to day length. The clock can also reset itself, so we can cope with the seasonal variation in day light hours and the trappings of 20th century living such as shift work and air travel. Not only humans have circadian rhythms. The eyes of marine molluscs, for example, show a correlation between perception of light and a circadian rhythm, as do the pineal glands of lizards and birds. The underlying clock that gives rise to these rhythms is dependent on feedback loops that regulate the expression of certain genes. Two animals in particular have given insight into the molecular mechanisms of internal clocks: the fungus *Neurospora crassa* and the fruit fly *Drosophila melanogaster*. Several components of molecular clocks have now been cloned and sequenced. In *Neurospora*, the *frq* gene was the first found to be associated with period length; then two more genes, *wc-1* and *wc-2*, were discovered in a strain of *Neurospora* that was blind to light. Both *wc-1* and *wc-2* are transcription factors that contain zinc fingers and transcriptional activation domains. Furthermore, these two proteins have PAS domains.

PAS domains were first identified in the *Drosophila* period clock protein PER, the vertebrate aryl hydrocarbon receptor nuclear translocator (ARNT), which

is involved in a cell's response to lowered oxygen levels, and the *Drosophila* single-minded protein (SIM1), involved in the regulation of development. Many proteins have since been found to have PAS domains, which have now been shown to mediate protein-protein interactions.

A series of recent papers have confirmed that there is a common pattern to molecular clocks that has been conserved across evolution, from fungi to mammals. Part of the pattern is that PAS domains glue proteins such as *wc-1* and *wc-2* together to form a complex that switches on other clock components, such as *frq*, as a part of the organism's response to light. The *frq* protein then feeds back to inhibit the action of *wc-1* and *wc-2*, thereby ultimately effecting its own expression. Signals from the environment, such as different light levels or temperature, could impact upon the loop to add more layers of regulation.

There are certain to be more feedback loops that are linked to this core component, because several observations have been made that do not quite fit this model, and it is not yet clear whether the clocks of plants or cyanobacteria will work in the same way. Perhaps these other cogs will be specific to different organisms, with only the "master clock", outlined here, being conserved across species. Time will tell.



Control of circadian rhythms at the molecular level We are just beginning to unravel the secrets of how circadian rhythms are controlled at the molecular level.

Several components of what might be the 'core' molecular clock have now been cloned and sequenced in a number of different organisms. In particular, efforts have focussed on the fungus *Neurospora crassa*, the fruit fly *Drosophila melanogaster*, and mammals such as mice and humans. Many of these components share a PAS dimerization domain and a basic helix-loop-helix DNA-binding motif.

Recent elegant work carried out in several laboratories has led to the current model, illustrated above. This proposes that proteins with PAS domains form heterodimers that bind DNA at specific sites, called E-boxes. E boxes are located in the promoter region of oscillator genes such as *Drosophila per* and *tim*. Binding of the heterodimer to the E box leads to the transcription of the oscillator genes. The proteins produced then feedback to ultimately inhibit their own production. Faded parts of the figure are speculative

Figure reproduced, with permission, from Dunlap, J. (1998) *Science* 280, 1548-1549.

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How might day length effect melatonin levels? [http://www.ncbi.nlm.nih.gov/Coffeebreak/CB3_Clock/PQ1.html]

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CLOCK protein from the fruit fly is one of the most recently discovered clock components. [http://www.ncbi.nlm.nih.gov/Coffeebreak/CB3_Clock/BQ.html]